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In vitro drug release of natamycin from β-cyclodextrin and 2-hydroxypropyl-β-cyclodextrin-functionalized contact lens materials

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Purpose: The antifungal agent natamycin can effectively form inclusion complexes with beta-cyclodextrin (β-CD) and 2-hydroxypropyl-β-cyclodextrin (HP-βCD) to improve the water solubility of natamycin by 16-fold and 152-fold, respectively (Koontz, J. Agric. Food. Chem. 2003). The purpose of this study was to develop contact lens materials functionalized with methacrylated β-CD (MβCD) and methacrylated HP-βCD (MHP-βCD), and to evaluate their ability to deliver natamycin *in vitro*. *Methods*: Model conventional hydrogel (CH) materials were synthesized by adding varying amounts of MβCD and MHP-βCD (0, 0.22, 0.44, 0.65, 0.87, 1.08% of total monomer weight) to a monomer solution containing 2-hydroxyethyl methacrylate (HEMA). Model silicone hydrogel (SH) materials were synthesized by adding similar concentrations of MβCD and MHP-βCD to N,N-dimethylacrylamide (DMAA)/10% 3-methacryloxypropyltris(trimethylsiloxy)silane (TRIS). The gels were cured with UV light, washed with ethanol and then, hydrated for 24 h (h). The model materials were then incubated with 2 mL of 100 μg/mL of natamycin in phosphate buffered saline (PBS) pH 7.4 for 48 h at room temperature. The release of natamycin from these materials in 2 mL of PBS, pH 7.4 at 32 ± 2 °C was monitored using UV–vis spectrophotometry at 304 nm over 24 h. *Results*: For both CH and SH materials, functionalization with MβCD and MHP-βCD improved the total amount of drugs released up to a threshold loading concentration, after which further addition of methacrylated CDs decreased the amount of drugs released ($p < 0.05$). The addition of CDs did not extend the drug release duration; the release of natamycin by all model materials reached a plateau after 12 h ($p < 0.05$). Overall, DMAA/10% TRIS materials released significantly more drug than HEMA materials ($p < 0.05$). The addition of MHP-βCD had a higher improvement in drug release than MβCD for both HEMA and DMAA/10% TRIS gels ($p < 0.05$). *Conclusions*: A high loading concentration of methacrylated CDs decreases overall drug delivery efficiency, which likely results from an unfavorable arrangement of the CDs within the polymer network leading to reduced binding of natamycin to the CDs. HEMA and DMAA/10% TRIS materials functionalized with MHP-βCD are more effective than those functionalized with MβCD to deliver natamycin.

Keywords: antifungal; contact lens; drug delivery; cyclodextrin; natamycin; silicone hydrogel

Introduction

There have been numerous cases of fungal eye infections associated with therapeutic and daily soft contact lens (CL) wear.[1–3] These infections occur as a result of fungal

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penetration of a compromised corneal epithelium,[4] and can lead to vision loss and blindness if left untreated.[5,6] In 2006, a worldwide outbreak of ocular fungal infections occurred as a result of a multipurpose CL solution ReNu MoistureLoc,[7–9] which has prompted further research into the management of ocular fungal infections.

In comparison with bacterial infections, there are few drugs available to treat ocular fungal infections. Fungi are eukaryotic and share similarities with human hosts, which make it difficult to identify unique drug targets.[10] Currently, natamycin (pimaricin) is the only commercially available and United States Food & Drug Administration (FDA) approved ocular antifungal.[11–13] The drug has low water solubility at physiological pH, and therefore is prescribed as a 5% ophthalmic suspension (Alcon, Fort Worth, TX).

However, in an eye drop form, the drug delivery is inefficient as the drugs are continuously diluted and washed away by tears,[14–16] or dispersed from the eye during blinking,[17] drainage,[14,16] or non-specific absorption.[14,16,18] As a result, it has been estimated that only 1–7% of the medication within an eye drop reaches the target ocular tissue,[17] while the remainder is subjected to systemic absorption.[19] To achieve therapeutic drug concentrations to treat ocular fungal infections, multiple dosing is typically required, sometimes as often as applications at hourly to two-hourly intervals.[20] This can lead to problems relating to patient compliance,[21–23] as well as the potential for drug overdosing.[24]

Drug delivery using CLs can potentially overcome several of the current limitations associated with eye drops. The post-lens tear film, formed as a result of placing a CL on the cornea, has limited tear exchange.[25,26] This is advantageous in regards to drug delivery, as drugs released from the CL into the tear film will have prolonged contact time with the cornea.[27] It has been estimated that over 50% of the drugs released from a CL can diffuse into the cornea, which is at least 35 times more efficient than eye drops.[28] Furthermore, the CL polymer can also act as a barrier and reservoir to provide sustained drug release over extended periods, which eliminates the need for multiple dosing.[29] CLs have already been used therapeutically as ‘bandage’ lenses to treat damaged corneas by preventing painful contact between the eyelids and the cornea, to enhance corneal healing, and to prevent further corneal complications.[30–32] Pharmaceuticals, including antibiotics and anti-inflammatory drugs are typically administered topically in tandem with these CLs.[33] Thus, the application of using CLs for antifungal ocular drug delivery would be an extension of an already accepted ophthalmic practice.

However, simple drug soaking with CLs does not produce optimal results, with drug release occurring rapidly within a few hours.[29] We have previously examined the drug delivery of natamycin from several commercial CLs, and observed a burst release within the first hour, followed by a plateau phase.[34] This is not surprising, as commercial CLs are only designed for refractive error correction, and further material modifications are necessary to improve drug delivery using these materials. Amongst the various approaches, the synthesis of a biomimetic material created through molecular imprinting methods has proven to be very successful in providing sustained drug release.[35] These hydrogels contain recognitive sites, which can specifically interact with the target drug.[35] However, one major limitation of this approach is that each material is specific to its target drug, and the same material cannot be used to provide the same effective delivery for other ophthalmic drugs.

One alternative approach would be to functionalize hydrogels with monomers capable of establishing interactions with a variety of drug molecules. In the pharmaceutical

field, cyclodextrins (CDs) have proven to be effective and versatile for a wide range of drug delivery applications, due to their ability to complex with a wide array of drugs.[36] CDs are a family of cyclic oligosaccharides with a hydrophilic outer surface and a lipophilic central cavity.[36] Commonly used CDs in the pharmaceutical field include α-CD, β-CD, and γ-CD, which are six-, seven-, eight-membered sugar rings, respectively.[36] Their unique chemical structure allows them to be used as complexing agents to increase the aqueous solubility of poorly water-soluble drugs, to increase both drug bioavailability and stability.[37] The use of β-cyclodextrin (βCD) and 2-hydroxypropyl-β-cyclodextrin (HP-βCD) has been suggested to improve the aqueous solubility of natamycin by 16- and 152-fold, respectively.[38] Thus, the incorporation of these molecules within a CL may allow for improved interactions between the CL and natamycin, leading to better drug delivery profiles. The purpose of the current study was to develop CL materials functionalized with β-CD and HP-βCD, and evaluate their ability to release natamycin *in vitro*.

Materials and methods

2-Hydroxyethyl methacrylate (HEMA), N,N-dimethylacrylamide (DMAA), ethylene glycol dimethacrylate (EGMDA), 3-methacryloxypropyltris(trimethylsiloxy)silane (TRIS) were purchased from Sigma–Aldrich (St. Louis, MO). Natamycin was purchased from EMD Millipore (Billerica, MA). Di-methacrylated β-cyclodextrin (MβCD) and di-methacrylated (2-hydroxypropyl)-β-cyclodextrin (MHP-βCD) were purchased from Specific Polymers (France). The molecular structure of MβCD and MHP-βCD is shown in Figure 1.

Equilibration of natamycin with MβCD and MHP-βCD

Various concentrations of the MβCD and MHP-βCD were dissolved in deionized (DI) water and vortexed for 10 min to determine the maximum water solubility of these compounds. Increasing amounts of natamycin was then added to these solutions, and allowed to equilibrate for 24 h to determine the maximum amount of natamycin that can be equilibrated (when equilibrated, the solution turns from opaque to clear).

CL materials

Model conventional hydrogel (CH) CL materials consisting of HEMA were synthesized based on a procedure previously reported by van Beek et al. [39] Model silicone hydrogel (SH) materials consisting of DMAA and TRIS were also prepared based on previously published work.[40] MβCD and MHP-βCD were dissolved in DI water (or ethanol) to concentrations of 50, 40, 30, 20, and 10 mg/mL. CH materials were synthesized by adding 400 µL of the above CD solution to 1.6 mL of HEMA. For the synthesis of SH materials, due to the immiscibility of water and TRIS, MβCD and MHP-βCD were dissolved in ethanol at similar concentrations and were added to 1.6 mL of DMAA/10% TRIS. Additionally, 95 µL (5% wt) of EGDMA (cross-linker) and 9.5 µL of 2-hydroxy-2-methypropiophenone (Irgacure1173, photoinitiatior, Sigma–Aldrich)

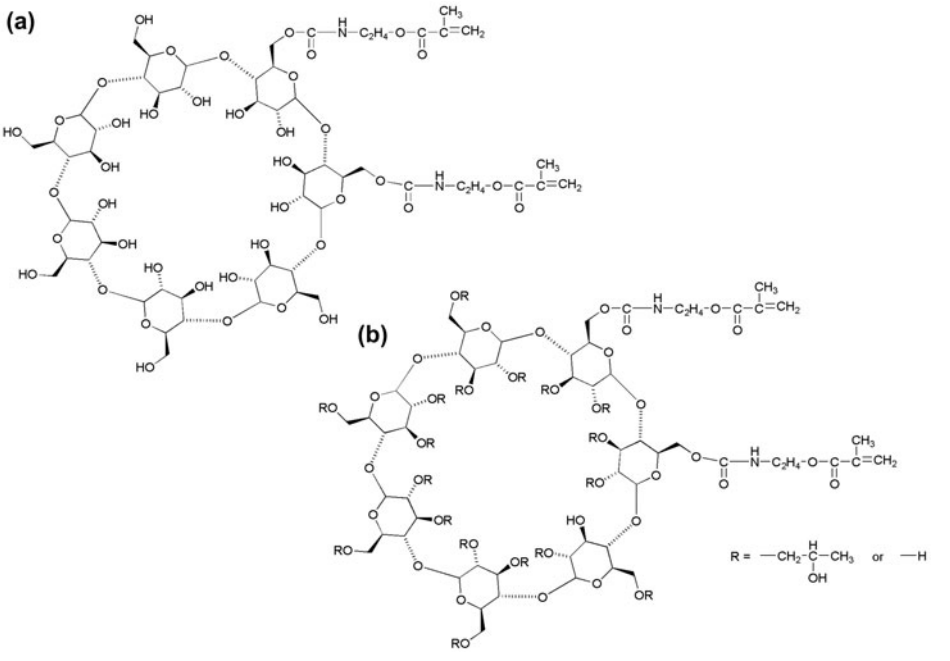

Figure 1. (a) Di-methacrylated β-cyclodextrin (MβCD) (MW = 1430 g/mol) and (b) di-methacrylated (2-hydroxypropyl)-β-cyclodextrin (MHP-βCD) (MW = 2710 g/mol).

were also added to the 2 mL monomer mixture. The resulting mixture was stirred for 5 min before being poured into a 42 mL aluminum weighing mold (Fisher Scientific, Pittsburg, PA). The mold was then placed inside the Dymax Ultraviolet (UV) Curing Chamber (Torrington, CT) and the gel was cured with UV light for 10 min (min). The molded gels were washed with ethanol and hydrated overnight in 100 mL of deionized (DI) water before they were cut into circular discs using a cork borer (1.45 cm diameter). The resulting gel discs (1.2 mm thickness) were dried overnight before further use.

Drug incubation and release

The above CL materials were soaked in a 2 mL suspension containing 100 mg/mL of natamycin in phosphate buffered saline (PBS), pH 7.4 over 48 h. Due to the turbidity of the suspension, the uptake of the drug into the CL material could not be monitored. After the uptake period, lenses were removed from the natamycin solution and briefly rinsed with PBS to remove any residual drug solution not sorbed onto the lens. The lenses were then partially dried on lens paper and placed into amber vials containing fresh 2 mL solution of PBS. The vials were incubated at 32 ± 1 °C with constant rotation over 24 h. The release of the drug was monitored using the SpectraMax M5 UV–vis Spectrophotometer at 304 nm by withdrawing 200 μL from the solution, which was then pipetted into a UV-Star Transparent Plate at specific time intervals t = 0, 1, 30 min, 1, 2, 4, 8, 12, 16, 24 h. After each measurement, the 200 μL sample solutions were pipetted back into their respective vials.

Water content

The wet weight (WW) of the lenses was measured using the Sartorius MA 100H (Goettingen, Germany). The lenses were then placed on a piece of lens paper and placed in a microwave for 2 min. Thereafter, the dry weight (DW) was measured using the Sartorius MA 100H. The water content (WC) was calculated using the following formula:

$$\mathrm{WC}(\%) = \frac{\mathrm{WW} - \mathrm{DW}}{\mathrm{WW}} \times 100$$

Statistical analysis

Statistical analysis was performed using Statistica 8 software (StatSoft, Tulsa, OK). All data are reported as mean ± standard deviation, unless otherwise stated. Repeated measures of analysis of variance were performed to determine the differences across various time points within the same lens material. An ANOVA was conducted to determine the differences between lens materials at each time point. Post-hoc Tukey multiple comparison tests were used when necessary. In all cases, statistical significance was considered significant for p values of <0.05. Graphs were plotted using GraphPad Prism 5 software (GraphPad, La Jolla, CA).

Results

Preliminary experiments in our lab with natamycin established that approximately 125 mg/mL of 2-hydroxypropyl-β-cyclodextrin in DI water could equilibrate completely with 2 mg/mL of natamycin after 24 h (h). This is comparable with results previously reported by Koontz and Marcy [38] However, the CD derivatives, MβCD and MHP-βCD could only be solubilized up to a concentration of 50 mg/mL in DI water. In addition, these CD derivatives could equilibrate only up to 500 μg/mL of natamycin after 48 h; the inclusion complex efficiency of MβCD and MHP-βCD was significantly less than the parent compound.[38]

For all CL materials, the drug release plateaued after 12 h, with neither MβCD nor MHP-βCD affecting the drug release duration ($p < 0.05$). However, functionalization with CD improved the total amount of drugs released for some materials ($p < 0.05$). As shown in Figure 2, the drug release for HEMA materials containing MβCD followed a trend in which an increase in CD beyond 0.22% of total polymer weight resulted in a reduction of drug release ($p < 0.05$). Similarly, HEMA materials containing 0.65% MHP-βCD had the highest drug release, and further increase in CD loading led to a decline in drug release (Figure 2(b)) ($p < 0.05$). The amount of natamycin released (μg/lens) as a function of time squared ($t^{1/2}$) for the first hour is plotted in Figure 2(c) (MβCD) and D(MHP-βCD).

For DMAA/10% TRIS materials, the amount of drug release correlated with increasing concentrations MβCD (Figure 3(a)) ($p < 0.05$), which was an exception to the observed trend. However, the functionalization of MHP-βCD with these materials continued to follow the trend, in which the highest drug release was observed at 0.48% MHP-βCD, and further CD addition resulted in a decrease in drug release (Figure 3(b)) ($p < 0.05$). Figure 3(c) (MβCD) and (d) (MHP-βCD) show the amount of natamycin released from DMAA/10% TRIS materials as a function of time squared ($t^{1/2}$).

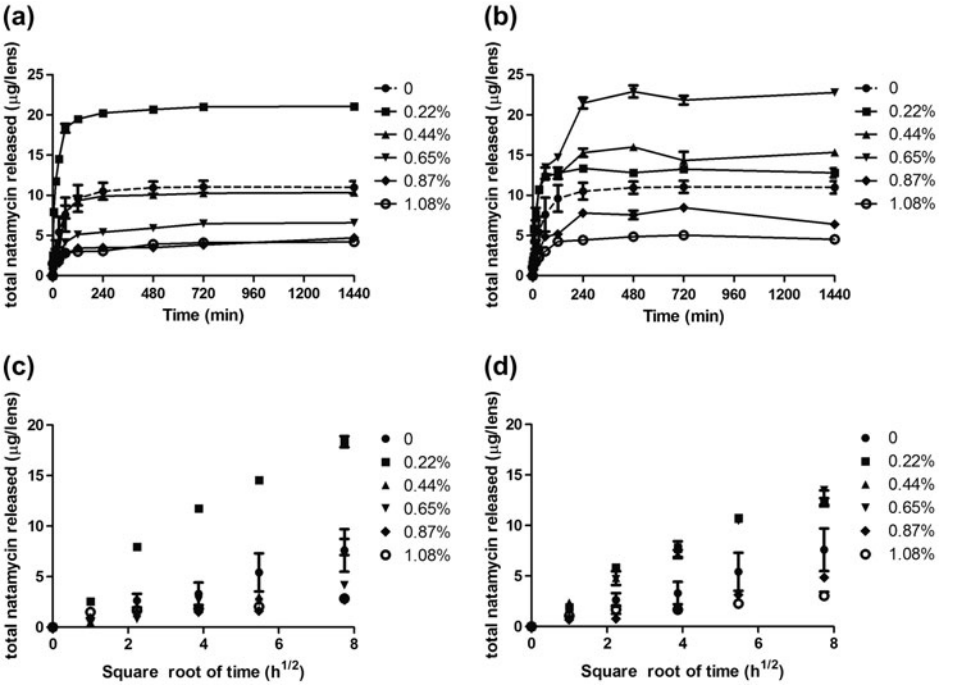

Figure 2. Total natamycin release (μg/lens) from HEMA gels functionalized with (a) MβCD (b) MHP-βCD after 24 h. The relationship between the amount of natamycin released (μg/lens) and $t^{1/2}$ for the first hour is plotted in (c) MβCD and (d) MHP-βCD. The values plotted are the mean ± standard deviation for three trials.

As a general trend, HEMA and DMAA/10% TRIS CL materials functionalized with MHP-βCD produced materials that could release higher amounts of natamycin compared to those functionalized with MβCD ($p < 0.05$). However, as shown in Figure 4(a) and (b), the percentage of CD in the polymer and the monomer composition also dictates which CD will be more effective. For instance, HEMA gels containing 0.22% MβCD released more drugs than the 0.22% MHP-βCD formulation ($p < 0.05$). DMAA/10% TRIS gels containing MβCD at 1.20% CD of polymer weight released more drugs than the MHP-βCD formulation ($p < 0.05$). Overall, the drug release was higher for DMAA/10% TRIS CL materials than HEMA materials ($p < 0.01$).

All model CL materials synthesized in this study were clear by visual inspection. When hydrated, DMAA/10% TRIS materials swelled more than HEMA-containing gels. Furthermore, as shown in Tables 1 and 2, DMAA/10% TRIS materials also had higher water content than HEMA gels ($p < 0.001$). The addition of either MβCD or MHP-βCD increased the equilibrium water content (EWC) of the lens material, in which increasing CD concentration resulted in higher EWC ($p < 0.05$). Overall, the addition of MβCD resulted in a higher EWC than MHP-βCD at similar concentrations ($p < 0.001$). Tables 1 and 2 summarize the properties of the CLs, and total amount of drug released by each gel after 8 and 24 h. The highest drug release after 24 h was observed for 0.48% MHP-βCD DMAA/10% TRIS (31.7 ± 1.2 μg after 24 h).

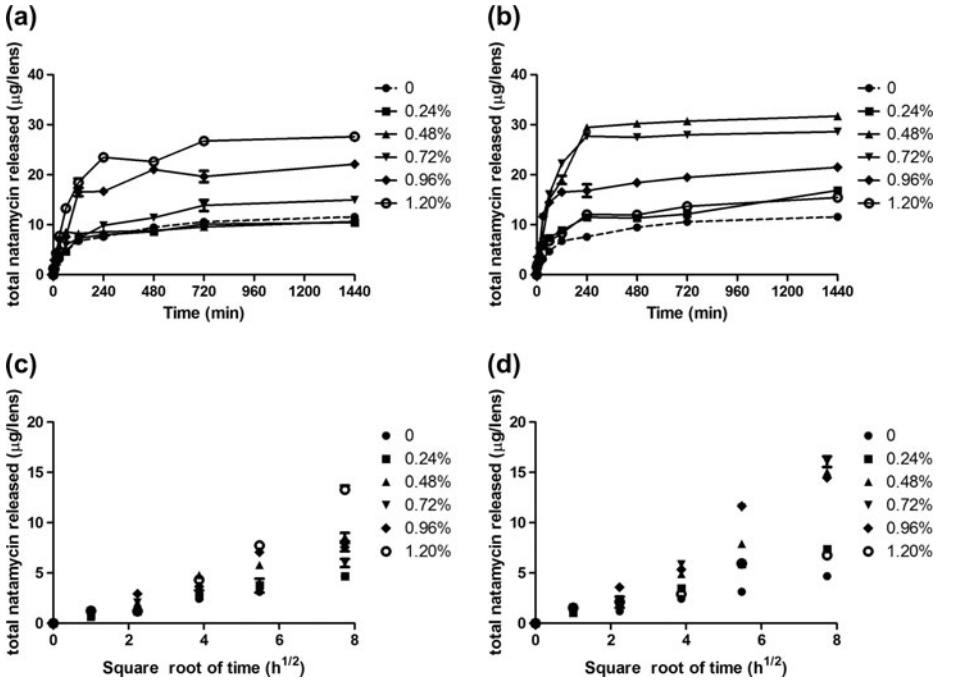

Figure 3. Total natamycin release (μg/lens) from DMAA/10% TRIS gels functionalized with (a) MβCD (b) MHP-βCD after 24 h. The relationship between the amount of natamycin released (μg/lens) and $t^{1/2}$ for the first hour is plotted in (c) MβCD and (d) MHP-βCD. The values plotted are the mean ± standard deviation for three trials.

Discussion

A previous study by Koontz and colleagues that used natamycin, [38] showed that the solubility of the antifungal can be increased 16-, 152-, and 73-fold when dissolved at the highest concentration of βCD (1.8% w/v), HP-βCD (50.0% w/v), and γCD (24.6% w/v). It would appear that the most effective CD to complex with natamycin would be HP-βCD, followed by γCD and βCD. However, one important factor to consider is the maximum water solubility of these CD, with βCD only having a maximum solubility at 18 mg/mL compared to HP-βCD at 500 mg/mL.[38] Thus, when CD solubility is also considered, βCD and HP-βCD have very similar inclusion complex efficiency with natamycin. At relatively lower CD concentrations below 20 mM, all three CDs were equally effective at complexing with natamycin.[38] As expected, the addition of two methacrylated chains to βCD and HP-βCD, to produce MβCD and MHP-βCD, resulted in compounds with a water solubility of only 50 mg/mL. This is almost a 10-fold decrease in solubility for HP-βCD, while the solubility for βCD improved by over 2-fold. However, as shown in Table 3, the ability of MβCD and MHP-βCD to complex with natamycin decreased in comparison to the parent compound.[38]

The incorporation of MβCD and MHP-βCD to HEMA and DMAA/10% TRIS model CL materials produced some unexpected results. The highest concentration of CDs initially loaded in the monomer mixture was 10 mg/mL (1.08–1.20% of total polymer weight). At this concentration, the amount of CDs forming inclusion

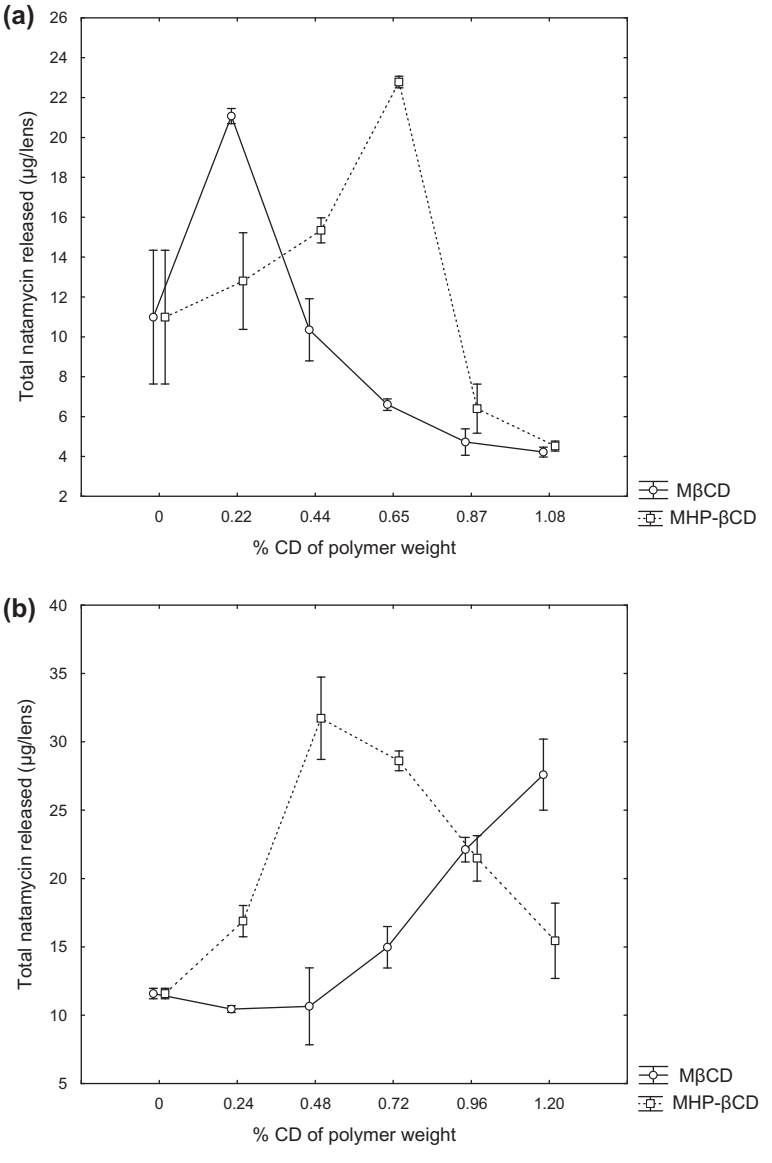

Figure 4. The relationship between total natamycin released (µg/lens) and the CD percent of total polymer weight for (a) HEMA and (b) DMAA/10% TRIS materials. The vertical bars denote 0.95 confidence intervals.

complexes with natamycin should increase linearly with increasing CD concentration.[38] However, only DMAA/10% TRIS gels incorporated with MβCD followed the expected trend. For HEMA gels incorporated with either MβCD or MHP-βCD above 0.22% and 0.65% of total polymer weight, the amount of natamycin released showed a reduction with increasing loading concentration of CDs. This was also observed for DMAA/10% TRIS materials functionalized with MHP-βCD at concentrations above 0.48%. This effect appears to be dependent on the monomer composition of the material, as well as the type of CD.

Table 1. Total amount of natamycin released after 8 and 24 h in 2 mL of PBS, pH 7.4 for HEMA materials. The values reported are the mean ± SD (n = 3).

Gel (HEMA)	MβCD (% by total polymer weight)	MHP-βCD (% by total polymer weight)	Water content (%)	Total drug release 8 h (μg/lens)	Total drug release 24 h (μg/lens)
1	0	0	15.5 ± 0.7	10.9 ± 1.3	11.0 ± 1.4
2	0.22	–	17.7 ± 0.1	20.6 ± 0.2	21.0 ± 0.2
3	0.44	–	21.1 ± 4.6	10.1 ± 0.56	10.4 ± 0.6
4	0.65	–	22.0 ± 0.5	6.0 ± 0.1	6.6 ± 0.1
5	0.87	–	23.9 ± 1.0	3.5 ± 0.1	4.7 ± 0.3
6	1.08	–	24.5 ± 3.0	3.9 ± 0.1	4.2 ± 0.1
7	–	0.22	15.6 ± 0.3	12.8 ± 0.6	12.8 ± 1.0
8	–	0.44	16.6 ± 0.6	16.0 ± 0.5	15.3 ± 0.3
9	–	0.65	20.8 ± 4.0	22.9 ± 1.3	22.8 ± 0.1
10	–	0.87	20.8 ± 4.0	7.5 ± 0.9	6.4 ± 0.5
11	–	1.08	18.4 ± 2.1	4.8 ± 0.2	4.5 ± 0.1

Table 2. Total amount of natamycin released after 8 and 24 h in 2 mL of PBS, pH 7.4 for DMAA/10% TRIS materials. The values reported are the mean ± SD (n = 3).

Gel (DMAA/ 10% TRIS)	MβCD (% by total polymer weight)	MHP-βCD (% by total polymer weight)	Water content (%)	Total drug release 8 h (μg/lens)	Total drug release 24 h (μg/lens)
12	0	0	24.1 ± 3.0	9.5 ± 1.0	11.6 ± 0.2
13	0.24	–	25.2 ± 0.5	8.6 ± 0.8	10.5 ± 0.1
14	0.48	–	30.1 ± 0.3	8.8 ± 0.4	10.7 ± 1.1
15	0.72	–	31.4 ± 1.7	11.4 ± 0.5	15.0 ± 0.6
16	0.96	–	39.0 ± 2.3	21.0 ± 1.0	22.1 ± 0.4
17	1.20	–	41.6 ± 2.0	22.6 ± 0.2	27.6 ± 1.0
18	–	0.24	21.8 ± 1.4	11.4 ± 0.5	16.9 ± 0.5
19	–	0.48	24.2 ± 2.1	30.2 ± 0.9	31.7 ± 1.2
20	–	0.72	27.1 ± 0.4	27.5 ± 0.6	28.6 ± 0.3
21	–	0.96	28.9 ± 1.0	18.4 ± 0.5	21.5 ± 0.7
22	–	1.20	33.0 ± 1.0	12.0 ± 0.4	15.4 ± 1.1

Table 3. Complexing efficiency of CDs with natamycin.

	CD Concentration (mg/mL)	Average molecular weight (g/mol)	CD Concentration (mM)	Estimated natamycin solubility (μg/mL)
βCD	18 (max)	1134.94	15.86	500 [38]
HP-βCD	50	1375.36	36.35	1250 [38]
γCD	50	1297.12	38.55	1000 [38]
MβCD	50	1430.00	35.00	500
MHP-βCD	50	2710.00	18.50	500

The underlying mechanism is not well understood and we propose the following hypothesis. Drugs are released from the CD gels from two sites: (1) non-specific sites formed randomly throughout the free space within the polymer and (2) specific sites formed by the CD. As the CD concentration increases, there is an increase in specific sites, resulting in increased drug release. However, due to volume constraints, as the number of specific sites increase beyond a threshold concentration, there is also a reduction in the number of non-specific sites. As a result, this offsets any increase in drug release provided by CDs. With a high CD concentration, the arrangement of the CD in the polymer becomes over saturated, in which their complexing centers are hindered by side chains, and become inaccessible to the drug.

We initially expected that the incorporation of CDs within the polymer, which could interact with the drug, would lead to a delayed and extended release of the drug from the polymer. However, all gels within this study released all the drug within the first 12 h, suggesting that time for drugs in CDS to equilibrate with PBS is about 12 h. This release duration is similar to what has been reported for two antifungals, naftifine, and terbinafine from hydrogels functionalized with βCD.[41] The drug release profile from the model CLs in this study also suggests a diffusion-controlled process, and the CDs did not significantly affect the rate of drug release. This suggests while the CDs can improve the total amount of drug that can be released, the rate of drug equilibration will remain similar to that of the control material.

In general, the incorporation of MHP-βCD with HEMA and DMAA/10% TRIS gels provides a higher amount of drug release than MβCD. However, the amount of drugs released is also dependent on the percent of CD in the polymer and the monomer composition of the gel. DMAA/10% TRIS materials released more drug than the HEMA-based materials. This trend has been observed previously in another study.[42] The mechanism is not clear, but it has been suggested that DMAA-based materials typically contain higher water content than HEMA-based materials, which helps facilitate the release of the drug from the polymer.[42]

An important factor in CL synthesis is to ensure a uniform distribution of the individual monomers by minimizing phase separation between monomers.[43] This can be accomplished by reducing the polymerization time via increasing the cross-linking density.[43] It has been reported that a composition of approximately 4% by total weight of a cross-linking agent is most optimal to decrease gelation time, and minimize phase separation effects.[43] In this study, a 5% EGDMA cross-linking density was used to ensure the distribution of CDs throughout the material. However, the typical amount of EGDMA material used in CL synthesis is approximately only 0.5% EGDMA.[42,44] By increasing the cross-linking density in a fixed volume, the resulting effect is a decrease in equilibrium water content (EWC).[45] Furthermore, EGDMA which is hydrophobic will also increase the overall hydrophobicity of the material. Previous studies report HEMA and DMAA materials with 0.5% EGDMA to contain approximately 30–35% and 44% EWC, respectively.[42] By increasing the concentration of EGDMA to 5% in this study, the EWC decreases for both HEMA and DMAA gels to 15.5 ± 0.7% and 24.1 ± 3.0%, respectively. The addition of either MβCD or MHP-βCD increased the EWC for all materials, with increasing CD content correlating to increasing EWC. Surprisingly, MβCD provided better improvement in EWC than MHP-βCD, although both of these compounds have similar water solubility, and HP-βCD is more water soluble than βCD. The mechanism as to why MβCD absorbs more water than MHP-βCD is unclear, however, we hypothesize that the additional

2-hydroxypropyl chains on MHP-βCD in a polymer network may occupy and displace water molecules in hydrophilic regions of the polymer.

One important limitation to consider when applying CDs to a CL is the amount of CDs that can be effectively functionalized into the polymer, which correlates to the amount of total drugs that can effectively form inclusion complexes with the material. Based on our results, 50 mg/mL of MβCD or MHP-βCD can effectively equilibrate up to 500 μg/mL of natamycin over 48 h. However, if we take into account the actual volume of CD present in a lens material, the amount of drug that can be complexed with the lens would only be approximately 20 μg per lens. Considering the clinical range where natamycin is effective against various fungi strains, such as *Fusarium spp* (MIC90 = 8 μg/mL) and *Candida spp* (MIC90 = 1–4 μg/mL), the amount of natamycin that can be complexed with the CD in these CLs may be too little.[46,47] Nonetheless, all gels in this study released enough drug in a 2 mL volume to meet the MIC90 for *Candida spp*, and gels 9,16–21 released enough drug to meet the MIC90 for *Fusarium spp*.

In conclusion, CD-functionalized CLs used in this study released more drug than the control CLs, with no significant differences between MβCD and MHP-βCD. When the loading of CDs increases beyond a threshold concentration, the arrangement of the CDs becomes crowded and the CD inclusion site becomes inaccessible to the drug. These CDs improve the EWC of both HEMA and DMAA/10% TRIS materials, with MβCD providing a better improvement. None of the gels studied released the drug for more than 12 h, but all model CLs released enough drug to meet the MIC90 for *Candida spp*. The application of MβCD and MHP-βCD could be extended to other hydrogels for the delivery of natamycin, and other antifungal drugs.

Abbreviations

CD	Cyclodextrin
MβCD	Methacrylated β-cyclodextrin
MHP-βCD	Methacrylated 2-hydroxypropyl-β-cyclodextrin
CL	Contact lens
SH	Silicone hydrogel
CH	Conventional hydrogel
EWC	Equilibrium water content
MIC90	Minimum inhibitory concentration for 90% of isolates

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References

[1] Hoflin-Lima AL, Roizenblatt R. Therapeutic contact lens-related bilateral fungal keratitis. CLAO J. 2002;28:149–150.
[2] Choi DM, Goldstein MH, Salierno A, Driebe WT. Fungal keratitis in a daily disposable soft contact lens wearer. CLAO J. 2001;27:111–112.
[3] Le Liboux MJ, Ibara SA, Quinio D, Moalic E. Fungal keratitis in a daily disposable soft contact lens wearer. J. Fr. Ophtalmol. 2004;27:401–403.

[4] Bharathi MJ, Ramakrishnan R, Vasu S, Meenakshi R, Palaniappan R. Epidemiological characteristics and laboratory diagnosis of fungal keratitis. A three-year study. Indian J Ophthalmol. 2003;51:315–321.
[5] Whitcher JP, Srinivasan M, Upadhyay MP. Corneal blindness: a global perspective. Bull. World Health Organaniz. 2001;79:214–221.
[6] Thomas PA. Current perspectives on ophthalmic mycoses. Clin. Microbiol. Rev. 2003;16:730–797.
[7] Elder BL, Bullock JD, Warwar RE, Khamis HJ, Khalaf SZ. Pan-antimicrobial failure of alexidine as a contact lens disinfectant when heated in Bausch & Lomb plastic containers. Eye Contact Lens: Sci. Clin. Pract. 2012;38:222–226.
[8] Yildiz EH, Abdalla YF, Elsahn AF, Rapuano CJ, Hammersmith KM, Laibson PR, Cohen EJ. Update on fungal keratitis from 1999 to 2008. Cornea. 2010;29:1406–1411.
[9] Bullock JD, Elder BL, Khamis HJ, Warwar RE. Effects of time, temperature, and storage container on the growth of Fusarium species. Arch. Ophthalmol. 2011;129:133–136.
[10] Li Y, Sun S, Guo Q, Ma L, Shi C, Su L, Li H. *In vitro* interaction between azoles and cyclosporin A against clinical isolates of Candida albicans determined by the chequerboard method and time-kill curves. J. Antimicrob. Chemother. 2008;61:577–585.
[11] O'Day DM. Selection of appropriate antifungal therapy. Cornea. 1987;6:238–245.
[12] Loh AR, Hong K, Lee S, Mannis M, Acharya NR. Practice patterns in the management of fungal corneal ulcers. Cornea. 2009;28:856–859.
[13] Kaur IP, Rana C, Singh H. Development of effective ocular preparations of antifungal agents. J. Ocul. Pharmacol. Ther. 2008;24:481–494.
[14] Chrai SS, Makoid MC, Eriksen SP, Robinson JR. Drop size and initial dosing frequency problems of topically applied ophthalmic drugs. J. Pharm. Sci. 1974;63:333–338.
[15] Friedlaender MH, Breshears D, Amoozgar B, Sheardown H, Senchyna M. The dilution of benzalkonium chloride (BAK) in the tear film. Adv. Ther. 2006;23:835–841.
[16] Shell JW. Pharmacokinetics of topically applied ophthalmic drugs. Surv. Ophthalmol. 1982;26:207–218.
[17] Ghate D, Edelhauser HF. Barriers to glaucoma drug delivery. J. Glaucoma. 2008;17: 147–156.
[18] Le Bourlais CL, Acar L, Zia H, Sado PA, Needham T, Leverge R. Ophthalmic drug delivery systems – recent advances. Prog. Retin Eye Res. 1998;17:33–58.
[19] Patton TF, Francoeur M. Ocular bioavailability and systemic loss of topically applied ophthalmic drugs. Am. J. Ophthalmol. 1978;85:225–229.
[20] Yolton DP. Antiinfective drugs. In: Bartlett JD, Jaanus SD, editors. Clinical ocular pharmacology. 4th ed. Woburn, MA: Butterworth-Heinemann; 2001. p. 219–264.
[21] Gurwitz JH, Glynn RJ, Monane M, Everitt DE, Gilden D, Smith N, Avorn J. Treatment for glaucoma: adherence by the elderly. Am. J. Public Health. 1993;83:711–716.
[22] Rotchford AP, Murphy KM. Compliance with timolol treatment in glaucoma. Eye. 1998;12:234–236.
[23] Winfield AJ, Jessiman D, Williams A, Esakowitz L. A study of the causes of non-compliance by patients prescribed eyedrops. Br. J. Ophthalmol. 1990;74:477–480.
[24] Trawick AB. Potential systemic and ocular side effects associated with topical administration of timolol maleate. J. Am. Optom. Assoc. 1985;56:108–112.
[25] Paugh JR, Stapleton F, Keay L, Ho A. Tear exchange under hydrogel contact lenses: methodological considerations. Invest. Ophthalmol. Visual Sci. 2001;42:2813–2820.
[26] Lin MC, Soliman GN, Lim VA, Giese ML, Wofford LE, Marmo C, Radke C, Polse KA. Scalloped channels enhance tear mixing under hydrogel contact lenses. Optom. Vision. Sci. 2006;83:874–878.
[27] Hehl EM, Beck R, Luthard K, Guthoff R, Drewelow B. Improved penetration of aminoglycosides and fluoroquinolones into the aqueous humour of patients by means of Acuvue contact lenses. Eur. J. Clin. Pharmacol. 1999;55:317–323.
[28] Li C-C, Chauhan A. Modeling ophthalmic drug delivery by soaked contact lenses. Ind. Eng. Chem. Res. 2006;45:3718–3734.
[29] Gulsen D, Chauhan A. Ophthalmic drug delivery through contact lenses. Invest. Ophthalmol. Visual Sci. 2004;45:2342–2347.
[30] Lim L, Tan DT, Chan WK. Therapeutic use of Bausch & Lomb PureVision contact lenses. CLAO J. 2001;27:179–185.

[31] Arora R, Jain S, Monga S, Narayanan R, Raina UK, Mehta DK. Efficacy of continuous wear PureVision contact lenses for therapeutic use. Cont. Lens Anterior Eye. 2004;27: 39–43.
[32] Foulks GN, Harvey T, Sundarraj CV. Therapeutic contact lenses: the role of high-Dk lenses. Ophthalmol. Clin. North Am. 2003;16:455–461.
[33] Karlgard CC, Jones LW, Moresoli C. Survey of bandage lens use in North America, October–December 2002. Eye Contact Lens: Sci. Clin. Pract. 2004;30:25–30.
[34] Phan CM, Subbaraman LN, Jones L. *In vitro* uptake and release of natamycin from conventional and silicone hydrogel contact lens materials. Eye Contact Lens: Sci. Clin. Pract. 2013;39:162–168.
[35] Mosbach K, Ramström O. The emerging technique of molecular imprinting and its future impact on biotechnology. Nat. Biotechnol. 1996;14:163–170.
[36] Arun Rasheed A, Kumar A, Sravanthi V. Cyclodextrins as drug carrier molecule: a review. Sci. Pharm. 2008;76:567–598.
[37] Loftsson T, Duchene D. Cyclodextrins and their pharmaceutical applications. Int. J. Pharm. 2007;329:1–11.
[38] Koontz JL, Marcy JE. Formation of natamycin: cyclodextrin inclusion complexes and their characterization. J. Agric. Food Chem. 2003;51:7106–7110.
[39] van Beek M, Weeks A, Jones L, Sheardown H. Immobilized hyaluronic acid containing model silicone hydrogels reduce protein adsorption. J. Biomater. Sci., Polym. Ed. 2008;19:1425–1436.
[40] Kim J, Conway A, Chauhan A. Extended delivery of ophthalmic drugs by silicone hydrogel contact lenses. Biomaterials. 2008;29:2259–2269.
[41] Machín R, Isasi JR, Vélaz I. β-Cyclodextrin hydrogels as potential drug delivery systems. Carbohydr. Polym. 2012;87:2024–2030.
[42] Phan CM, Subbaraman L, Liu S, Gu F, Jones L. *In vitro* uptake and release of natamycin Dex-b-PLA nanoparticles from model contact lens materials. J. Biomater. Sci., Polym. Ed. 2014;25:18–31.
[43] Raj WRP, Sasthav M, Cheung HM. Microcellular polymeric materials from microemulsions: control of microstructure and morphology. J. Appl. Polym. Sci. 1993;47:499–511.
[44] Garrett Q, Laycock B, Garrett RW. Hydrogel lens monomer constituents modulate protein sorption. Invest. Ophthalmol. Visual Sci. 2000;41:1687–1695.
[45] Martinez AW, Caves JM, Ravi S, Li W, Chaikof EL. Effects of crosslinking on the mechanical properties, drug release and cytocompatibility of protein polymers. Acta Biomater. 2014;10:26–33.
[46] Haller I, Plempel M. Experimental *in vitro* and *in vivo* comparison of modern antimycotics. Curr. Med. Res. Opin. 1977;5:315–327.
[47] Xu Y, Pang G, Gao C, Zhao D, Zhou L, Sun S, Wang B. *In vitro* comparison of the efficacies of natamycin and silver nitrate against ocular fungi. Antimicrob. Agents Chemother. 2009;53:1636–1638.